PATENT CLAIMS

Sub B1

1. An enzymatic test kit for the diagnosis of tuberculosis and other mycobacterial infections in humans and animals by determination of the activity of alanine dehydrogenase (E.C. 1.4.1.1), comprising L-alanine, nicotinamide adenine dinucleotide (oxidised form; NAD^+), phenazine methosulphate (PMS) and nitroblue tetrazolium chloride (NBT).

Sub B2

2. A method for the diagnosis of tuberculosis and other mycobacterial infections of humans and animals, ***characterised*** in that the activity of alanine dehydrogenase (E.C. 1.4.1.1.) is measured with an enzymatic test kit according to claim 1.

3. A method according to claim 2, ***characterised*** in that

(i) possible tuberculosis pathogens, such as *M. tuberculosis*, are isolated,

(ii) a crude cell extract is made,

(iii the extract is incubated in solution and

(iv) the absorption is measured.

4. A method according to claim 2 and/or 3, ***characterised*** in that clinical samples, such as body fluids, are subjected directly to tuberculosis diagnosis and the alanine dehydrogenase activity is measured.

5. A method according to claim 2, ***characterised*** in that cells, strains and/or species of disease-causing organisms (mycobacteria) are differentiated from non-virulent cells and strains.

6. A method according to claim 5, ***characterised*** in that cells, strains and/or species of disease-causing organisms of the *M. tuberculosis* complex are identified and differentiated.

7. A method according to any one of the preceding claims, ***characterised*** in that the method is carried out in the presence of substances that inhibit tuberculosis and other mycobacterial infections of humans and animals and those inhibiting substances are optionally recovered.

sub a2

8. A method according to any one of the preceding claims, ***characterised*** in that it is carried out

(i) to control epidemics and/or

(ii) after vaccinations (vaccination follow-up) in humans and animals.

9. A DNA sequence selected from the following group or other partial sequences of the alanine dehydrogenase gene of *M. tuberculosis* (Fig. 2.5):

Name	Sequence	Orientation
AlaDH-F1	5'-ATGCGCGTCGGTATTCCG-3'	forward
AlaDH-F1+	5'-GCGCGTCGGTATTCCGACCG-3'	forward
AlaDH-F2	5'-GAGACCAAAACAACGAA-3'	forward
AlaDH-F4	5'-GAATTCCCATCAGCAATCTTGCAGA-3'	forward
AlaDH-F5	5'-GCCCCGATGAGCGAAGTC-3'	forward
AlaDH-F6	5'-GGGGCCGTCCTGGTGCC-3'	forward
AlaDH-F7	5'-GACGTCGACCTACGCGCTGAC-3'	forward
AlaDH-R1	5'-CTCGGTGAACGGCACCCC-3'	reverse
AlaDH-R2	5'-GGCCAGCACGCTGGCGGG-3'	reverse
AlaDH-R3	5'-CACCCGTTCGGACAGTAA-3'	reverse
AlaDH-R4	5'-CGCGGCCGACATCATCGC-3'	reverse
AlaDH-R5	5'-GGCCGACATCATCGCTTCCC-3'	reverse
AlaDH-R6	5'-CGAGACTAATTTGGGTGCCTTGGC-3'	reverse
AlaDH-R7	5'-ATTTGGGTGCCTTGGC-3'	reverse
AlaDH-RM	5'-GGCGGCGAGTCGACCGGC-3'	reverse

and partial sequences thereof and sequences that are hybridisable therewith preferably at a temperature of at least 20°C and especially at a concentration of 1M NaCl and a temperature of at least 25°C, for the diagnosis of tuberculosis and other mycobacterial infections in humans and animals.

10. The use of a DNA sequence according to claim 9 for the diagnosis of tuberculosis and other mycobacterial infections in humans and animals.

11. A method according to claim 10, ***characterised*** in that a DNA sequence according to claim 9 is used

(i) for hybridisation,

(ii) for culture confirmation of isolated strains and/or

(iii) for chromosomal fingerprinting, and cells, strains and/or types of mycobacteria are determined and differentiated and/or are used for the diagnosis of mycobacterial infections.

12. A method according to claim 10 or 11, ***characterised*** in that cells, strains and/or species of virulent mycobacteria are differentiated from non-virulent cells, strains and/or species.

13. A method according to claim 10, ***characterised*** in that cells, strains and/or species of the *M. tuberculosis* complex and other mycobacteria

(i) are isolated,

(ii) crude or purified genomic DNA or RNA is recovered,

(iii) a fragment that is identical or virtually identical to the sequence of the alanine dehydrogenase gene of *M. tuberculosis* (Fig. 2.3) is identified, preferably by amplification using a DNA sequence according to claim 9 as a primer sequence, after which digestion is carried out with a restriction enzyme, especially Bg1II, and gel

electrophoresis of the digested amplified DNA is carried out and/or the DNA sequence of the amplified DNA is determined.

14. A method according to claim 2 and/or 10, ***characterised*** in that a clinical sample is used directly and diagnosed for tuberculosis in humans and animals.

15. A method according to claim 2 and/or 10, ***characterised*** in that the method is carried out in the presence of substances that inhibit tuberculosis or mycobacterial infections of humans and animals and inhibiting substances determined are recovered or made.

16. A method according to claim 10, ***characterised*** in that it is used

(i) in antimycobacterial chemotherapy,

(ii) in the control of epidemics and/or

(iii) after vaccinations (vaccination follow-up) in humans and animals.